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(57) Abstract

The invention provides human proteins having secretory signal sequences and DNAs coding for these proteins as well as eucaryotic cells forming said proteins by secretory expression. All of the proteins are secreted outside the cells and exist in the extracellular liquid or on the cell membrane, so that they are considered to be proteins controlling the proliferation and the differentiation of the cells. Accordingly, the proteins can be employed as pharmaceuticals or as antigens for preparing antibodies against said proteins. Furthermore, said DNAs can be utilized for expression of said proteins in large amounts. Eucaryotic cells wherein expression vectors of said cDNAs are introduced can be utilized for secretory production of the proteins encoded by said cDNAs.

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DESCRIPTION

Human Proteins Having Secretory Signal Sequences and cDNAs Encoding these Proteins

TECHNICAL FIELD

The present invention relates to human proteins having secretory signal sequences and DNAs coding for these proteins as well as eucaryotic cells forming said proteins by secretory expression. The proteins of the present invention can be employed as pharmaceuticals or as antigens for preparing antibodies against said proteins. The human cDNAs of the present invention can be utilized as probes for the gene diagnosis and gene sources for the gene therapy. Furthermore, the cDNAs can be utilized as gene sources for large-scale production of the proteins encoded by said cDNAs. Animal cells wherein expression vectors of said cDNAs are introduced can be utilized for secretory production of the proteins encoded by said cDNA.

BACKGROUND ART

Cells secrete many proteins outside the cells. These secretory proteins play important roles for the proliferation control, the differentiation induction, the material transportation, the biological protection, etc. in the cells. Different form intracellular proteins, the secretory proteins exert their actions outside the cells, whereby they can be administered in the intracorporeal manner such as the injection or the drip, so that there are hidden potentialities as medicines.

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In fact, a number of human secretory proteins such as interferons, interleukins, erythropoietin, thrombolytic agents, etc. have been currently employed as medicines. In addition, secretory proteins other than those described above have been undergoing clinical trials to develop as pharmaceuticals. Because it has been conceived that the human cells still produce many unknown secretory proteins, availability of these secretory proteins as well as genes coding for them is expected to lead to development of novel pharmaceuticals utilizing these proteins.

Heretofore, such secretory proteins have been obtained by a method comprising isolation and purification of the target protein from a large amount of the blood or a cell culture supernatant by using a biological activity as an indicator, determination of its primary structure followed by cloning of the corresponding cDNA on the basis of the information on the thus-obtained amino acid sequence, and production of a recombinant protein using said cDNA. However, the contents of the secretory proteins are generally so low that the isolation and purification are difficult in many cases. On the other hand, secretory proteins and type-1 membrane proteins possess hydrophobic sequences, defined as secretory signal sequences, consisting of about 20 amino acid residues at the amino acid termini (the N-termini). Therefore, the cloning of genes coding for the secretory proteins or type-1 membrane proteins is expected to be carried out by using the presence or absence of these secretory signal sequences as indicators.

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DISCLOSURE OF INVENTION

The object of the present invention is to provide proteins having secretory signal sequences and DNAs coding for these proteins as well as eucaryotic cells forming said proteins by secretory expression.

As the result of intensive studies, the present inventors have been successful in selective cloning of cDNAs having secretory signal sequences from a human full-length cDNA bank, thereby completing the present invention. In other words, the present invention provides human proteins having secretory signal sequences, namely proteins containing any of the amino acid sequences represented by Sequence No. 1 to Sequence 4. Moreover, the present invention provides DNAs coding for the above-mentioned proteins, exemplified by cDNAs containing any of the base sequences represented by Sequence Nos. 5 to 9, 11, 13, and 15, as well as transformation eucaryotic cells that are capable of expressing said cDNAs.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01738.

Figure 2: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01766.

Figure 3: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by

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clone HP01842.

Figure 4: A figure depicting the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10484.

BEST MODE FOR CARRYING OUT THE INVENTION

The proteins of the present invention can be obtained, for example, by a method for isolation from human organs, cell lines, etc., a method for preparation of peptides by the chemical synthesis, or a method for production with the recombinant DNA technology using the DNAs coding for the human secretory proteins of the present invention, wherein the method for obtainment by the recombinant DNA technology is employed preferably. For instance, in vitro expression can be achieved by preparation of an RNA by in vitro transcription from a vector having one of cDNAs of the present invention, followed by in vitro translation using this RNA as a template. Also, recombination of the translation region into a suitable expression vector by the method known in the art leads to expression of a large amount of the encoded protein by using prokaryotic cells such as *Escherichia coli*, *Bacillus subtilis*, etc., and eucaryotic cells such as yeasts, insect cells, mammalian cells, etc.

In the case in which a protein of the present invention is expressed by prokaryotic cells such as *Escherichia coli* etc., a recombinant expression vector bearing the translation region in the cDNA of the present invention is constructed in an expression vector having an origin, a promoter, a ribosome-binding site, a

cDNA-cloning site, a terminator etc., which can be replicated in the prokaryotic cells, and, after transformation of the host cells with said expression vector, the thus-obtained transformant is incubated, whereby the protein encoded by said cDNA can be produced on a large scale in the prokaryotic cells. In this case, a maturation protein can be obtained by carrying out the expression with inserting an initiation codon in the translation region wherein the secretory signal sequence is removed. Alternatively, a fusion protein with another protein can be expressed. Only a protein portion coding for said cDNA can be obtained by cleavage of said fusion protein with a suitable protease.

In the case in which a protein of the present invention is expressed by secretion in eucaryotic cells, the protein of the present invention can be produced by extracellular secretion, when the translation region of said cDNA is subjected to recombination to an expression vector for eucaryotic cells that has a promoter, a splicing region, a poly(A) addition site, etc., followed by introduction into the eucaryotic cells. The expression vector is exemplified by pKA1, pED6dpc2, pCDM8, pSVK3, pMSG, pSVL, pBK-CMV, pBK-RSV, EBV vector, pRS, pYES2, and so on. Examples of eucaryotic cells to be used in general include mammalian culture cells such as simian kidney cells COS7, Chinese hamster ovary cells CHO, etc., budding yeasts, fission yeasts, silkworm cells, *Xenopus laevis* egg cells, and so on, but any eucaryotic cells may be used, provided that they are capable of forming the present proteins by secretory expression. The expression vector can be introduced in the eucaryotic cells by methods known in the art such as the

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electroporation method, the potassium phosphate method, the liposome method, the DEAE-dextran method, and so on.

After one of the proteins of the present invention is expressed in prokaryotic cells or eucaryotic cells, the objective protein can be isolated from the culture and purified by a combination of separation procedures known in the art. Such examples include treatment with a denaturing agent such as urea or a surface-active agent, sonication, enzymatic digestion, salting-out or solvent precipitation, dialysis, centrifugation, ultrafiltration, gel filtration, SDS-PAGE, isoelectric focusing, ion-exchange chromatography, hydrophobic chromatography, affinity chromatography, reverse phase chromatography, and so on.

The proteins of the present invention include peptide fragments (more than 5 amino acid residues) containing any partial amino acid sequence in the amino acid sequences represented by Sequence No. 1 to Sequence No. 4. These peptide fragments can be utilized as antigens for preparation of antibodies. Hereupon, the proteins of the present invention are secreted in the form of maturation proteins outside the cells, after the signal sequences are removed. Therefore, these maturation proteins shall come within the scope of the present invention. The N-terminal amino acid sequences of the maturation proteins can be easily identified by using the method for the cleavage-site determination in a signal sequence [Japanese Patent Kokai Publication No. 1996-187100]. Furthermore, many secretory proteins undergo the processing after the secretion to be converted to the active forms. These activated proteins or peptides shall come within the scope of the present

invention. When sugar chain-binding sites are present in the amino acid sequences, expression in appropriate eucaryotic cells affords proteins wherein sugar chains are added. Accordingly, such proteins or peptides wherein sugar chains are added shall come within the scope of the present invention.

The DNAs of the present invention include all DNAs coding for the above-mentioned proteins. Said DNAs can be obtained by using a method by chemical synthesis, a method by cDNA cloning, and so on.

The cDNAs of the present invention can be cloned, for example, from cDNA libraries of the human cell origin. These cDNA are synthesized by using as templates poly(A)$^+$ RNAs extracted from human cells. The human cells may be cells delivered from the human body, for example, by the operation or may be the culture cells. The cDNAs can be synthesized by using any method selected from the Okayama-Berg method [Okayama, H. and Berg, P., Mol. Cell. Biol. 2: 161-170 (1982)], the Gubler-Hoffman method [Gubler, U. and Hoffman, J. Gene 25: 263-269 (1983)], and so on, but it is preferred to use the capping method [Kato, S. et al., Gene 150: 243-250 (1994)], as exemplified in Examples, in order to obtain a full-length clone in an effective manner.

The primary selection of one of the cDNAs coding for the human proteins having secretory signal sequences is carried out by sequencing of a partial base sequence of a cDNA clone selected at random from cDNA libraries, sequencing of the amino acid sequence encoded by the base sequence, and recognition of the presence or absence of a hydrophobic site in the resulting N-

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terminal amino acid sequence region. Next, the secondary selection is carried out by determination of the whole sequence by the sequencing and the protein expression by in vitro translation. Ascertainment of cDNAs of the present invention for encoding the proteins having secretory signal sequences is carried out by using the signal sequence detection method [Yokoyama-Kobayashi, M. et al., Gene 163: 193-196 (1995)]. In other words, the ascertainment for a coding portion of an inserted cDNA fragment to function as a signal sequence is provided by fusing a cDNA fragment coding for the N-terminus of the target protein with a cDNA coding for the protease domain of urokinase and then expressing the resulting cDNA in COS7 cells to detect the urokinase activity in the cell culture medium.

The cDNAs of the present invention are characterized by containing either of the base sequences represented by Sequence No. 5 to Sequence No. 8 or the base sequences represented by Sequence Nos. 9, 11, 13 and 15. Table 1 summarizes the clone number (HP number), the cells affording the cDNA, the total base number of the cDNA, and the number of the amino acid residues of the encoded protein, for each of the cDNAs.

Table 1

Sequence No.	HP No.	Cell	Number of bases	Number of amino amino acids
1, 5, 9	HP01738	Stomach Cancer	665	167
2, 6, 10	HP01766	PMA-U937	1414	172
3, 7, 11	HP01842	PMA-U937	596	144
4, 8, 12	HP10484	PMA-U937	1234	220

Hereupon, the same clones as the cDNAs of the present invention can be easily obtained by screening of the cDNA libraries constructed from the human cell lines and human tissues utilized in the present invention by the use of an oligonucleotide probe synthesized on the basis of the cDNA base sequence described in any of Sequence Nos. 9, 11 and 13.

In general, the polymorphism due to the individual difference is frequently observed in human genes. Accordingly, any cDNA that is subjected to insertion or deletion of one or plural nucleotides and/or substitution with other nucleotides in Sequence Nos. 5 to 9, 11, 13 and 15 shall come within the scope of the present invention.

In a similar manner, any protein that is formed by these modifications comprising insertion or deletion of one or plural amino acids and/or substitution with other amino acids shall come within the scope of the present invention, as far as the protein possesses the activity of any protein having the amino acid sequences represented by Sequence No. 1 to Sequence No. 4.

The cDNAs of the present invention include cDNA fragments (more than 10 bp) containing any partial base sequence in the base sequences represented by Sequence No. 5 to Sequence No. 8 or in the base sequences represented by Sequence Nos. 9, 11, 13 and 15. For instance, as illustrated in Examples, the portion coding for the secretory signal sequence can be utilized as means to secrete an optionally selected protein outside the cells by fusing with a cDNA encoding another protein. Also, DNA fragments consisting of a sense chain and an anti-sense chain shall come within this

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scope. These DNA fragments can be utilized as the probes for the gene diagnosis.

In addition to the activities and uses described above, the polynucleotides and proteins of the present invention may exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for

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selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodiesusing DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these

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binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell

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differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation,

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Kruisbeek, A.M. and Shevach, E.M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6-Nordan, R. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark,S.C. and Turner, K.J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell

interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial orfungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal

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infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy

in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to

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anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Sciencė 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function in vivo on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms.

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Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the commoncold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

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Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected ex vivo with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result

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in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection in vivo.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class IIα chain protein and an MHC class IIβ chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

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The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J.J. and Brunswick, M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

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Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al.,

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Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and

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proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-

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hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and

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cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have

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prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral

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neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for

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promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the

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protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to

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tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

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Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (includinghereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and

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their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory

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process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of ytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth

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Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an

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immune response against such protein or another material or entity which is cross-reactive with such protein.

Examples

The present invention is embodied in more detail by the following examples, but this embodiment is not intended to restrict the present invention. The basic operations and the enzyme reactions with regard to the DNA recombination are carried out according to the literature ["Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory, 1989]. Unless otherwise stated, restrictive enzymes and a variety of modification enzymes to be used were those available from TAKARA SHUZO. The manufacturer's instructions were used for the buffer compositions as well as for the reaction conditions, in each of the enzyme reactions. The cDNA synthesis was carried out according to the literature [Kato, S. et al., Gene 150: 243-250 (1994)].

(1) Preparation of Poly(A)$^+$ RNA

The histiocyte lymphoma cell line U937 (ATCC CRL 1593) stimulated by phorbol ester and tissues of stomach cancer delivered by the operation were used for human cells to extract mRNAs. The cell line was incubated by a conventional procedure.

After about 1 g of the human cells was homogenized in 20 ml of a 5.5 M guanidinium thiocyanate solution, a total mRNA was prepared according to the literature [Okayama, H. et al., "Method in Enzymology", Vol. 164, Academic Press, 1987]. This was subjected to chromatography on oligo(dT)-cellulose column washed with a 20 mM Tris-hydrochloride buffer solution (pH 7.6), 0.5 M

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NaCl, and 1 mM EDTA to obtain a poly(A)$^+$ RNA according to the above-described literature.

(2) Construction of cDNA Library

Ten micrograms of the above-mentioned poly(A)$^+$ RNA were dissolved in a 100 mM Tris-hydrochloride buffer solution (pH 8), one unit of an RNase-free, bacterial alkaline phosphatase was added, and the reaction was run at 37℃ for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in a solution containing 50 mM sodium acetate (pH 6), 1 mM EDTA, 0.1% 2-mercaptoethanol, and 0.01% Triton X-100. Thereto was added one unit of a tobacco-origin acid pyrophosphatase (Epicentre Technologies) and a total 100 µl volume of the resulting mixture was reacted at 37℃ for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in water to obtain a solution of a decapped poly(A)$^+$ RNA.

The decapped poly(A)$^+$ RNA and 3 nmol of a chimeric DNA-RNA oligonucleotide (5'-dG-dG-dG-dG-dA-dA-dT-dT-dC-dG-dA-G-G-A-3') were dissolved in a solution containing 50 mM Tris-hydrochloride buffer solution (pH 7.5), 0.5 mM ATP, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 25% polyethylene glycol, whereto was added 50 units of T4RNA ligase and a total 30 µl volume of the resulting mixture was reacted at 20℃ for 12 hours. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in water to obtain a chimeric-oligo-capped poly(A)$^+$ RNA.

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After digestion of vector pKA1 (Japanese Patent Kokai Publication No. 1992-117292) developed by the present inventors with KpnI, about 60 dT tails were added using a terminal transferase. A vector primer to be used below was prepared by digestion of this product with EcoRV to remove a dT tail at one side.

After 6 μg of the previously-prepared chimeric-oligo-capped poly(A)$^+$ RNA was annealed with 1.2 μg of the vector primer, the resulting product was dissolved in a solution containing 50 mM Tris-hydrochloride buffer solution (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, and 1.25 mM dNTP (dATP + dCTP + dGTP + dTTP), 200 units of a reverse transcriptase (GIBCO-BRL) were added, and the reaction in a total 20 μl volume was run at 42℃ for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in a solution containing 50 mM Tris-hydrochloride buffer solution (pH 7.5), 100 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol. Thereto were added 100 units of EcoRI and a total 20 μl volume of the resulting mixture was reacted at 37℃ for one hour. After the reaction solution was subjected to phenol extraction, followed by ethanol precipitation, the resulting pellet was dissolved in a solution containing 20 mM Tris-hydrochloride buffer solution (pH 7.5), 100 mM KCl, 4 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, and 50 μg/ml of the bovine serum albumin. Thereto were added 60 units of an *Escherichia coli* DNA ligase and the resulting mixture was reacted at 16℃ for 16 hours. To the reaction solution were added 2 μl of 2 mM dNTP, 4 units of *Escherichia coli* DNA polymerase I, and 0.1 unit of *Escherichia coli* RNase H and

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the resulting mixture was reacted at 12℃ for one hour and then at 22℃ for one hour.

Next, the cDNA-synthesis reaction solution was used for transformation of *Escherichia coli* DH12S (GIBCO-BRL). The transformation was carried out by the electroporation method. A portion of the transformant was sprayed on the 2xYT agar culture medium containing 100 μg/ml ampicillin and the mixture was incubated at 37℃ overnight. A colony formed on the agar medium was picked up at random and inoculated on 2 ml of the 2xYT culture medium containing 100 μg/ml ampicillin. After incubation at 37℃ overnight, the culture mixture was centrifuged to separate the mycelia, from which a plasmid DNA was prepared by the alkaline lysis method. The plasmid DNA was subjected to double digestion with EcoRI and NotI, followed by 0.8% agarose gel electrophoresis, to determine the size of the cDNA insert. Furthermore, using the thus-obtained plasmid as a template, the sequence reaction was carried out by using an M13 universal primer labeled with a fluorescent dye and a Taq polymerase (a kit of Applied Biosystems) and then the product was examined with a fluorescent DNA sequencer (Applied Biosystems) to determine an about 400-bp base sequence at the 5'-terminus of the cDNA. The sequence data were filed as the homo/protein cDNA bank database.

(3) Selection of cDNAs Encoding Proteins Having Secretory Signal Sequences

A base sequence registered in the homo/protein cDNA bank was converted to three frames of amino acid sequences and the presence or absence of an open reading frame (ORF) beginning from

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the initiation codon was examined. Then, the selection was made for the presence of a signal sequence that is characteristic to a secretory protein at the N-terminus of the portion encoded by the ORF. These clones were sequenced from the both 5' and 3' directions by the use of the deletion method using exonuclease III to determine the whole base sequence. The hydrophobicity/hydrophilicity profiles were obtained for proteins encoded by the ORF by the Kyte-Doolittle method [Kyte, J. & Doolittle, R. F., J. Mol. Biol. 157: 105-132 (1982)] to examine the presence or absence of a hydrophobic region. In the case in which there is not a hydrophobic region of a putative transmembrane domain in the amino acid sequence of an encoded protein, this protein was judged as a membrane protein that did not possess a secretory protein or a transmembrane domain.

(4) Functional Verification of Secretory Signal Sequence

It was verified by the method described in the literature [Yokoyama-Kobayashi, M. et al., Gene 163: 193-196 (1995)] that the N-terminal hydrophobic region in the secretory protein clone candidate obtained in the above-mentioned steps functions as a secretory signal sequence. First, the plasmid containing the target cDNA was cleaved at an appropriate restriction enzyme site existing at the downstream of the portion expected for encoding the secretory signal sequence. In the case in which this restriction site was a protruding 5'-terminus, the site was blunt-ended by the Klenow treatment. Digestion with HindIII was further carried out and a DNA fragment containing the SV40 promoter and a cDNA encoding the secretory signal sequence at the downstream

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of the promoter was separated by agarose gel electrophoresis. The resulting fragment was inserted between HindIII in pSSD3 (DDBJ/EMBL/GenBank Registration No. AB007632) and a restriction enzyme site selected so as to match with the urokinase-coding frame, thereby constructing a vector expressing a fusion protein of the secretory signal sequence of the target cDNA and the urokinase protease domain.

After *Escherichia coli* (host: JM109) bearing the fusion-protein expression vector was incubated at 37℃ for 2 hours in 2 ml of the 2xYT culture medium containing 100 μg/ml of ampicillin, the helper phage M13KO7 (50 μl) was added and the incubation was continued at 37 ℃ overnight. A supernatant separated by centrifugation underwent precipitation with polyethylene glycol to obtain single-stranded phage particles. These particles were suspended in 100 μl of 1 mM Tris-0.1 mM EDTA, pH 8 (TE). Also, there were used as controls suspensions of single-stranded phage particles prepared in the same manner from pSSD3 and from the vector pKA1-UPA containing a full-length cDNA of urokinase [Yokoyama-Kobayashi, M. et al., Gene 163: 193-196 (1995)].

The culture cells originating from the simian kidney, COS7, were incubated at 37℃ in the presence of 5% CO_2 in the Dulbecco's modified Eagle's culture medium (DMEM) containing 10% fetal calf albumin. Into a 6-well plate (Nunc Inc., 3 cm in the well diameter) were inoculated 1×10^5 COS7 cells and incubation was carried out at 37℃ for 22 hours in the presence of 5% CO_2. After the culture medium was removed, the cell surface was washed with a phosphate

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buffer solution and then washed again with DMEM containing 50 mM Tris-hydrochloric acid (pH 7.5) (TDMEM). To the resulting cells was added a suspension of 1 µl of the single-stranded phage suspension, 0.6 ml of the DMEM culture medium, and 3 µl of TRANSFECTAM™ (IBF Inc.) and the resulting mixture was incubated at 37℃ for 3 hours in the presence of 5% CO_2. After the sample solution was removed, the cell surface was washed with TDMEM, 2 ml per well of DMEM containing 10% fetal calf albumin was added, and the incubation was carried out at 37℃ for 2 days in the presence of 5% CO_2.

To 10 ml of 50 mM phosphate buffer solution (pH 7.4) containing 2% bovine fibrinogen (Miles Inc.), 0.5% agarose, and 1 mM calcium chloride were added 10 units of human thrombin (Mochida Pharmaceutical Co., Ltd.) and the resulting mixture was solidified in a plate of 9 cm in diameter to prepare a fibrin plate. Ten microliters of the culture supernatant of the tansfected COS7 cells were spotted on the fibrin plate, which was incubated at 37℃ for 15 hours. The diameter of the thus-obtained clear circle was taken as an index for the urokinase activity. Table 2 shows the restriction enzyme site used for cutting the cDNA fragment from each clone, the restriction enzyme site used for cleavage of pSSD3, and the presence or absence of a clear circle. Except for the case in which pSSD3 was used as the control, each of the samples formed a clear circle to identify that urokinase was secreted in the culture medium. In other words, it has been indicated that each of the cDNA fragments codes for the amino acid sequence that functions as the secretory signal sequence.

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Table 2

HP No.	Restriction enzyme site		Clear circle
	cDNA*	Vector	
HP01738	BglII (K)	PmaCI	+
HP01766	PvuII	EcoRV	−
HP01842	PstI (T)	SmaI	+
HP10484	BalI	EcoRV	+
pKA1−UPA			+
pSSD3			−

* (K) and (T) mean that the cDNA was blunt-ended by the Klenow treatment, after cleavage with the restriction enzyme, and by the T4DNA-polymerase treatment, after cleavage with the restriction enzyme, respectively.

(5) Protein Synthesis by In Vitro Translation

The plasmid vector bearing the cDNA of the present invention was used for in vitro transcription/translation with a T$_7$T rabbit reticulocyte lysate kit (Promega). In this case, [^{35}S]methionine was added to label the expression product with a radioisotope. Each of the reactions was carried out according to the protocols attached to the kit. Two micrograms of the plasmid was reacted at 30℃ for 90 minutes in a total 25 μl volume of the reaction solution containing 12.5 μl of T$_7$T rabbit reticulocyte lysate, 0.5 μl of a buffer solution (attached to kit), 2 μl of an amino acid mixture (methionine-free), 2 μl of [^{35}S]methionine (Amersham) (0.37 MBq/μl), 0.5 μl of T7RNA polymerase, and 20 U of RNasin. To 3 μl of the resulting reaction solution was added 2 μl of the SDS sampling buffer (125 mM Tris-hydrochloric acid buffer, pH 6.8,

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120 mM 2-mercaptoethanol, 2% SDS solution, 0.025% bromophenol blue, and 20% glycerol) and the resulting mixture was heated at 95℃ for 3 minutes and then subjected to SDS-polyacrylamide gel electrophoresis. The molecular weight of the translation product was determined by carrying out the autoradiograph.

(6) Expression by COS7

Escherichia coli bearing the expression vector of the protein of the present invention was infected with helper phage M13KO7 and single-stranded phage particles were obtained by the above-mentioned procedure. The thus-obtained phage was used for introducing each expression vector in the culture cells originating from the simian kidney, COS7. After incubation at 37℃ for 2 days in the presence of 5% CO_2, the incubation was continued for one hour in the culture medium containing [^{35}S]cystine or [^{35}S]methionine. Concentration of the culture medium, followed by subjecting to SDS-PAGE, allowed to observe the presence of a band wherein the protein of the present invention was formed by the secretory expression, which did not exist in the culture supernatant from the COS7 cells only. The molecular weights of the expression products were as follows: HP01738, 17kDa; HP01842, 32kDa; HP10484, 27kDa.

(7) Clone Examples

<HP01738> (Sequence Nos. 1, 5, and 9)

Determination of the whole base sequence of the cDNA insert of clone HP01738 obtained from cDNA libraries of the human stomach cancer revealed the structure consisting of a 58-bp 5′-nontranslation region, a 504-bp ORF, and a 103-bp 3′-

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nontranslation region. The ORF codes for a protein consisting of 167 amino acid residues and there existed a hydrophobic region of a putative secretory signal sequence at the N-terminal. Figure 1 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 19 kDa that was almost consistent with the molecular weight of 18,176 predicted from the ORF. Application of the (-3,-1) rule, a method for predicting the cleavage site in the secretory signal sequence, allows to expect that the maturation protein starts from isoleucine at position 19.

The search of the protein data base by using the amino acid sequence of the present protein revealed that the protein was analogous to the rat zymogen granule protein (NBRF Accession No. S42924). Table 3 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the rat zymogen granule protein (RN). Therein, the marks of -, *, and . represent a gap, an amino acid residue identical with the protein of the present invention, and an amino acid residue analogous to the protein of the present invention, respectively. The both proteins possessed a homology of 84.5%. Hereupon, the present protein had a C-terminus longer by 18 amino acid residues.

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Table 3

```
HS MLTVALLALLCASASGNAIQARSSSYSGEYGSGGGKRFSHSGNQLDGPITALRVRVNTYY
   **..***.*******.*.**.**********.  ******************.*.*** **
RN MLAIALLVLLCASASANSIQSRSSSYSGEYGGKGGKRFSHSGNQLDGPITAIRIRVNRYY
HS IVGLQVRYGKVWSDYVGGRNGDLEEIFLHPGESVIQVSGKYKWYLKKLVFVTDKGRYLSF
   *.*******.********..  . ******************** *.*.*.*********.*
RN IIGLQVRYGTVWSDYVGGNR-ETEEIFLHPGESVIQVSGKYKSYVKQLIFVTDKGRYLPF
HS GKDSGTSFNAVPLHPNTVLRFISGRSGSLIDAIGLHWDVYPTSCSRC
   ****************************
RN GKDSGTSFNAVPLHPNTVLRFISGRSGSA
```

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. AA296957) in EST, but any of the sequences was shorter than the present cDNA and was not found to contain the initiation codon.

<HP01766> (Sequence Nos. 2, 6, and 11)

Determination of the whole base sequence of the cDNA insert of clone HP01766 obtained from cDNA libraries of human lymphoma cell line U937 revealed the structure consisting of a 96-bp 5'-nontranslation region, a 519-bp ORF, and a 799-bp 3'-nontranslation region. The ORF codes for a protein consisting of 172 amino acid residues and there existed a hydrophobic region of a putative secretory signal sequence at the N-terminal. Figure 2 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 26

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kDa that was somewhat larger than the molecular weight of 19,205 predicted from the ORF. Application of the (-3,-1) rule, a method for predicting the cleavage site in the secretory signal sequence, allows to expect that the maturation protein starts from serine at position 24.

The search of the protein data base by using the amino acid sequence of the present protein revealed that the protein was analogous to the *Xenopus laevis* putative secretory protein XAG (GenBank Accession No. U76752). Table 4 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the *Xenopus laevis* putative secretory protein XAG (XL). Therein, the marks of -, *, and . represent a gap, an amino acid residue identical with the protein of the present invention, and an amino acid residue analogous to the protein of the present invention, respectively. The both proteins possessed a homology of 34.5%.

Table 4

```
HS                          METRPRLGATCLLGFSFLLLVISSDGHNGLGKGFGDHIH
                                                *...* ..*..*.*. *.
XL MQAGLSLVCLVLLCSALGEAVLKKPKKQAGTTDTKTDQEPAPIKTKGLKTLDRGWGESIE
HS W-RTLEDGKKEAAASGLPLMVIIHKSWCGACKALKPKFAESTEISELSH-NFVMVNLEDE
   * .* *.*  .* ... ***** * .  *  . ***  *...    .*.. .*.*.**
XL WVQTYEEGLAKARENNKPLMVIHHLEDCPYSIALKKAFVADRMAQKLAQEDFIMLNL--V
HS EEPKDEDFSPDGGYIPRILFLDPSGKVHPEIINENGNPSYKYFYVSAEQVVQGMKEAQER
   .   **. **** *.**..*.*** .*.... .  **  * *   .  ... .**.*..
XL HPVADENQSPDGHYVPRVIFIDPSLTVRSDLKGRYGNKMYAYDADDIPELITNMKKAKSF
HS LTGDAFRKKHLEDEL
   *...
XL LKTEL
```

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. R23553) in EST, but many sequences were not distinct and the same ORF as that in the present cDNA was not found.

<HP01842> (Sequence Nos. 3, 7, and 13)

Determination of the whole base sequence of the cDNA insert of clone HP01842 obtained from cDNA libraries of human lymphoma U937 revealed the structure consisting of an 84-bp 5'-nontranslation region, a 435-bp ORF, and a 77-bp 3'-nontranslation region. The ORF codes for a protein consisting of 144 amino acid residues and there existed a hydrophobic region of a putative secretory signal sequence at the N-terminal. Figure 3 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 17 kDa that was almost consistent with the molecular weight of 16,158 predicted from the ORF. Application of the (-3,-1) rule, a method for predicting the cleavage site in the secretory signal sequence, allows to expect that the maturation protein starts from glutamic acid at position 25.

The search of the protein data base by using the amino acid sequence of the present protein revealed that the protein was analogous to the human putative calcium-binding protein (NBRF Accession No. JS0027). Table 5 shows the comparison of the amino acid sequence between the human protein of the present invention (HP) and the human putative calcium-binding protein (CB). Therein,

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the marks of - and * represent a gap and an amino acid residue identical with the protein of the present invention, respectively. The both proteins possessed a homology of 98.8%. Hereupon, the present protein had an N-terminus longer by 64 amino acid residues.

Table 5

```
HS MRSLLRTPFLCGLLWAFCAPGARAEEPAASFSQPGSMGLDKNTVHDQEHIMEHLEGVINK
HS PEAEMSPQELQLHYFKMHDYDGNNLLDGLELSTAITHVHKEEGSEQAPLMSEDELINIID
      ************************************************************
CB      MSPQELQLHYFKMHDYDGNNLLDGLELSTAITHVHKEEGSEQAPLMSEDELINIID
HS GVLRDDDKNNDGYIDYAEFAKSLQ
   ******* ****************
CB GVLRDDD-NNDGYIDYAEFAKSLQ
```

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. AA132163) in EST, but since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10484> (Sequence Nos. 4, 8, and 15)

Determination of the whole base sequence of the cDNA insert of clone HP10484 obtained from cDNA libraries of human lymphoma cell line U937 revealed the structure consisting of a 52-bp 5′-nontranslation region, a 663-bp ORF, and a 519-bp 3′-nontranslation region. The ORF codes for a protein consisting of 220 amino acid residues and there existed a hydrophobic region

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of a putative secretory signal sequence at the N-terminal. Figure 4 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 26 kDa that was almost consistent with the molecular weight of 24,074 predicted from the ORF. Application of the (-3,-1) rule, a method for predicting the cleavage site in the secretory signal sequence, allows to expect that the maturation protein starts from arginine at position 32. In addition, there exist in the amino acid sequence of this protein three sites where N-glycosylation is likely to occur (Asn-Glu-Thr at position 160, Asn-Ile-Thr at position 193, and Asn-Val-Thr at position 216).

The search of the protein data base using the amino acid sequence of the present protein has not revealed the presence of any known protein having an analogy. Also, the search of the GenBank using the base sequences of the present cDNA has revealed the presence of sequences that possessed a homology of 90% or more (for example, Accession No. AA309845) in EST, but any of the sequences was shorter than the present cDNAs and was not found to contain the initiation codon.

INDUSTRIAL APPLICABILITY

The present invention provides human proteins having secretory signal sequences and DNAs coding for these proteins as well as eucaryotic cells forming said proteins by secretory expression. All of the proteins of the present invention are secreted outside the cells and exist in the extracellular liquid

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or on the cell membrane, so that they are considered to be proteins controlling the proliferation and the differentiation of the cells. Accordingly, the proteins of the present invention can be employed as pharmaceuticals or as antigens for preparing antibodies against said proteins. Furthermore, said DNAs can be utilized for expression of said proteins in large amounts. Eucaryotic cells wherein expression vectors of said cDNAs are introduced can be utilized for secretory production of the proteins encoded by said cDNAs.

The present invention also provides genes corresponding to the polynucleotide sequences disclosed herein. "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which cDNA polynucleotide sequences are derived and may include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" is a gene that has been separated from the adjacent coding sequences, if any, present in the genome of the organism from which the gene was isolated.

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Organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein are provided. The desired change in gene expression can be achieved through the use of antisense polynucleotides or ribozymes that bind and/or cleave the mRNA transcribed from the gene (Albert and Morris, 1994, Trends Pharmacol. Sci. 15(7): 250-254; Lavarosky et al., 1997, Biochem. Mol. Med. 62(1): 11-22; and Hampel, 1998, Prog. Nucleic Acid Res. Mol. Biol. 58: 1-39; all of which are incorporated by reference herein). Transgenic animals that have multiple copies of the gene(s) corresponding to the polynucleotide sequences disclosed herein, preferably produced by transformation of cells with genetic constructs that are stably maintained within the transformed cells and their progeny, are provided. Transgenic animals that have modified genetic control regions that increase or reduce gene expression levels, or that change temporal or spatial patterns of gene expression, are also provided (see European Patent No. 0 649 464 B1, incorporated by reference herein). In addition, organisms are provided in which the gene(s) corresponding to the polynucleotide sequences disclosed herein have been partially or completely inactivated, through insertion of extraneous sequences into the corresponding gene(s) or through deletion of all or part of the corresponding gene(s). Partial or complete gene inactivation can be accomplished through insertion, preferably followed by imprecise excision, of transposable elements (Plasterk, 1992, Bioessays 14(9): 629-633; Zwaal et al., 1993, Proc. Natl. Acad. Sci. USA 90(16): 7431-7435;

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Clark et al., 1994, Proc. Natl. Acad. Sci. USA 91(2): 719-722; all of which are incorporated by reference herein), or through homologous recombination, preferably detected by positive/negative genetic selection strategies (Mansour et al., 1988, Nature 336: 348-352; U.S. Patent Nos. 5,464,764; 5,487,992; 5,627,059; 5,631,153; 5,614, 396; 5,616,491; and 5,679,523; all of which are incorporated by reference herein). These organisms with altered gene expression are preferably eukaryotes and more preferably are mammals. Such organisms are useful for the development of non-human models for the study of disorders involving the corresponding gene(s), and for the development of assay systems for the identification of molecules that interact with the protein product(s) of the corresponding gene(s).

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at least 25% (more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most

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preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are proteins and protein fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. As used herein, a "species homologue" is a protein or polynucleotide with a different species of origin from that of a given protein or polynucleotide, but with significant sequence similarity to the given protein or polynucleotide, as determined by those of skill in the art. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous, or related to that encoded by the polynucleotides.

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides disclosed herein.

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The present invention also includes polynucleotides capable of hybridizing under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

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Table 6

Stringency Condition	Polynucleotide Hybrid	Hybrid Length (bp)‡	Hybridization Temperature and Buffer†	Wash Temperature and Buffer†
A	DNA : DNA	≥50	65℃; 1×SSC -or- 42℃; 1×SSC,50% formamide	65℃; 0.3×SSC
B	DNA : DNA	<50	T_B*; 1×SSC	T_B*; 1×SSC
C	DNA : RNA	≥50	67℃; 1×SSC -or- 45℃; 1×SSC,50% formamide	67℃; 0.3×SSC
D	DNA : RNA	<50	T_D*; 1×SSC	T_D*; 1×SSC
E	RNA : RNA	≥50	70℃; 1×SSC -or- 50℃; 1×SSC,50% formamide	70℃; 0.3×SSC
F	RNA : RNA	<50	T_F*; 1×SSC	T_F*; 1×SSC
G	DNA : DNA	≥50	65℃; 4×SSC -or- 42℃; 4×SSC,50% formamide	65℃; 1×SSC
H	DNA : DNA	<50	T_H*; 4×SSC	T_H*; 4×SSC
I	DNA : RNA	≥50	67℃; 4×SSC -or- 45℃; 4×SSC,50% formamide	67℃; 1×SSC
J	DNA : RNA	<50	T_J*; 4×SSC	T_J*; 4×SSC
K	RNA : RNA	≥50	70℃; 4×SSC -or- 50℃; 4×SSC,50% formamide	67℃; 1×SSC
L	RNA : RNA	<50	T_L*; 2×SSC	T_L*; 2×SSC
M	DNA : DNA	≥50	50℃; 4×SSC -or- 40℃; 6×SSC,50% formamide	50℃; 2×SSC
N	DNA : DNA	<50	T_N*; 6×SSC	T_N*; 6×SSC
O	DNA : RNA	≥50	55℃; 4×SSC -or- 42℃; 6×SSC,50% formamide	55℃; 2×SSC
P	DNA : RNA	<50	T_P*; 6×SSC	T_P*; 6×SSC
Q	RNA : RNA	≥50	60℃; 4×SSC -or- 45℃; 6×SSC,50% formamide	60℃; 2×SSC
R	RNA : RNA	<50	T_R*; 4×SSC	T_R*; 4×SSC

‡ : The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

† : SSPE (1×SSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH7.4) can be substituted for SSC (1×SSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

*T_B - T_R : The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10℃ less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(℃)=2(#of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(℃)=81.5 + 16.6($\log_{10}$[Na^+]) + 0.41 (%G+C) - (600/N), where N is the number of bases in the hybrid, and [Na^+] is the concentration of sodium ions in the hybridization buffer ([Na^+] for 1×SSC=0.165M).

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BNSDOCID: <WO 9918204A2 1 >

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and Current Protocols in Molecular Biology, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

Preferably, each such hybridizing polynucleotide has a length that is at least 25% (more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

CLAIMS

1. A protein comprising any of the amino acid sequences represented by Sequence Nos. 1 to 4.

2. A DNA coding for the protein according to Claim 1.

3. A cDNA comprising any of the base sequences represented by Sequence Nos. 5 to 8.

4. The cDNA according to Claim 3 comprising any of the base sequences represented by Sequence Nos. 9, 11, 13 and 15.

5. A vector capable of expressing the DNA or cDNA according to any of Claims 2 to 4 in in vitro translation or an eucaryotic cell.

6. A transformation eucaryotic cell capable of expressing the DNA or cDNA according to any of Claims 2 to 4 to produce the protein according to Claim 1.

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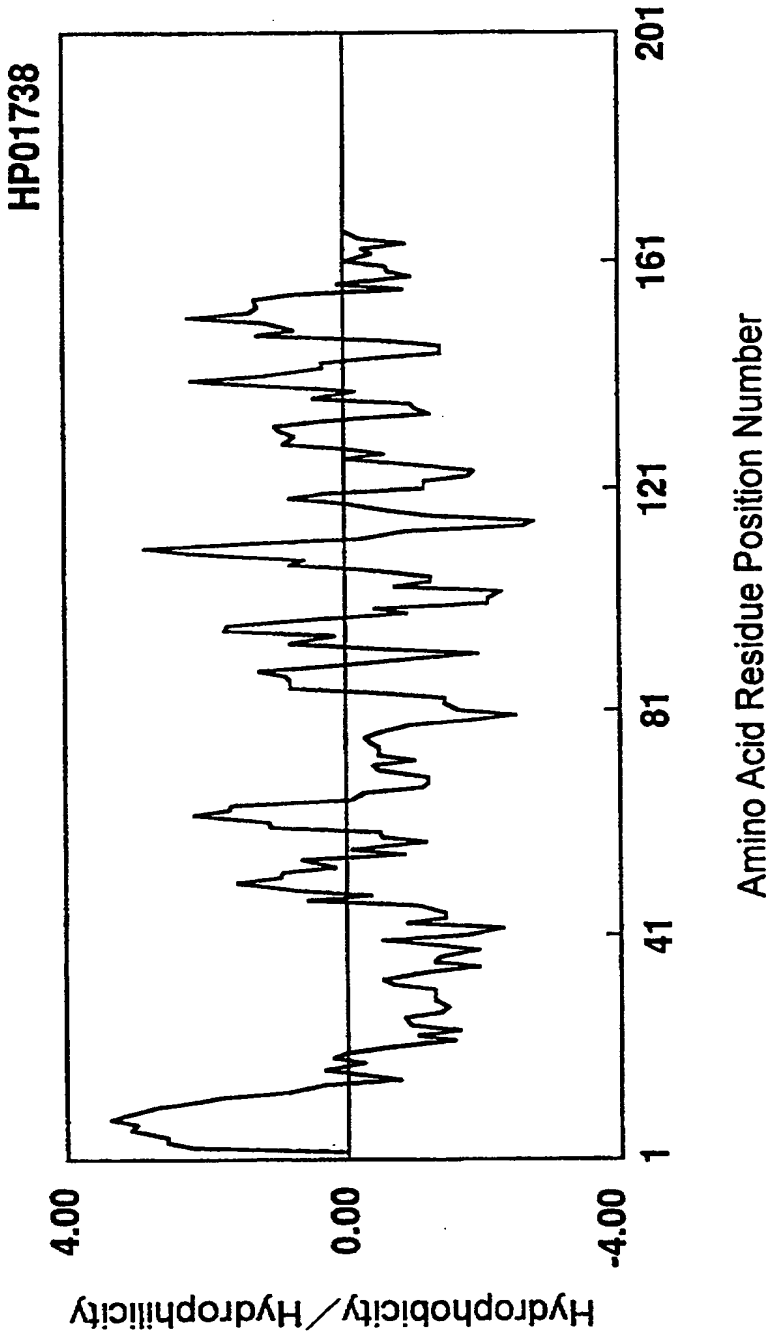

Fig. 1

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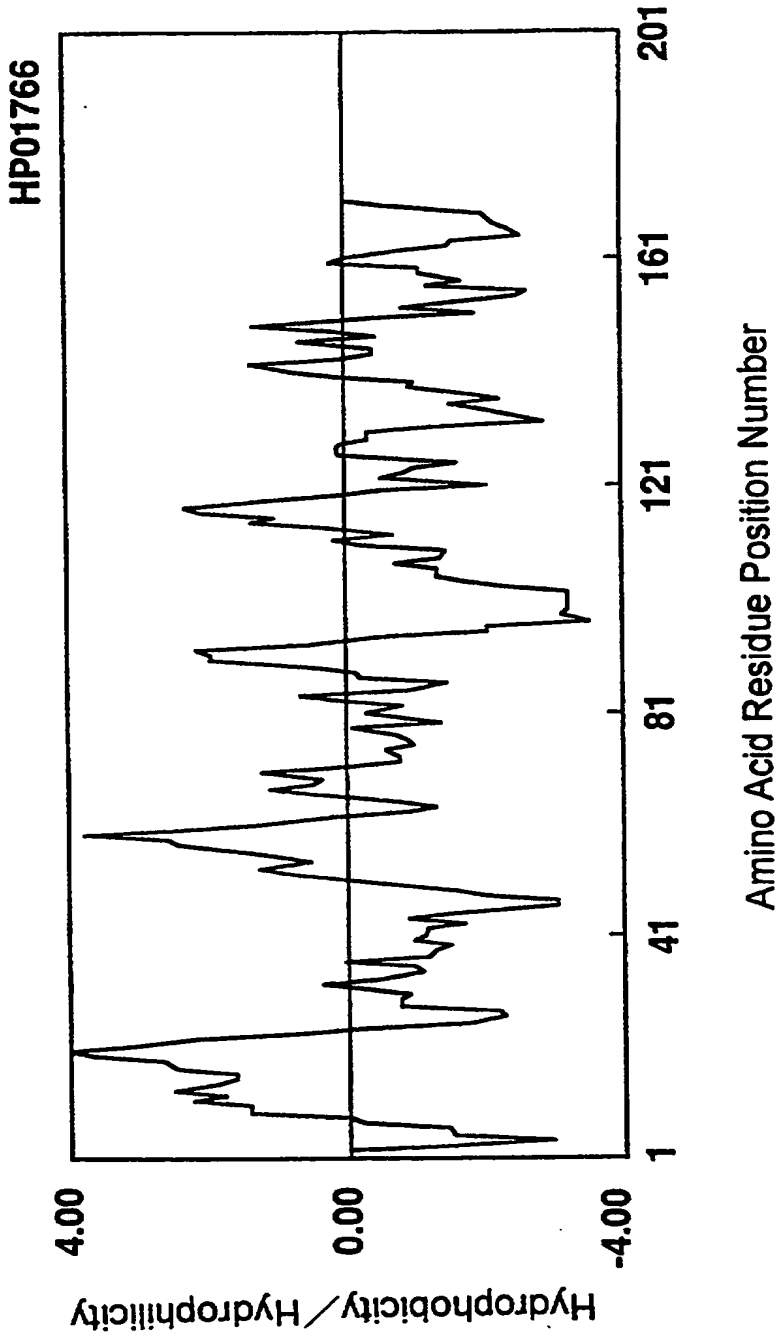

Fig. 2

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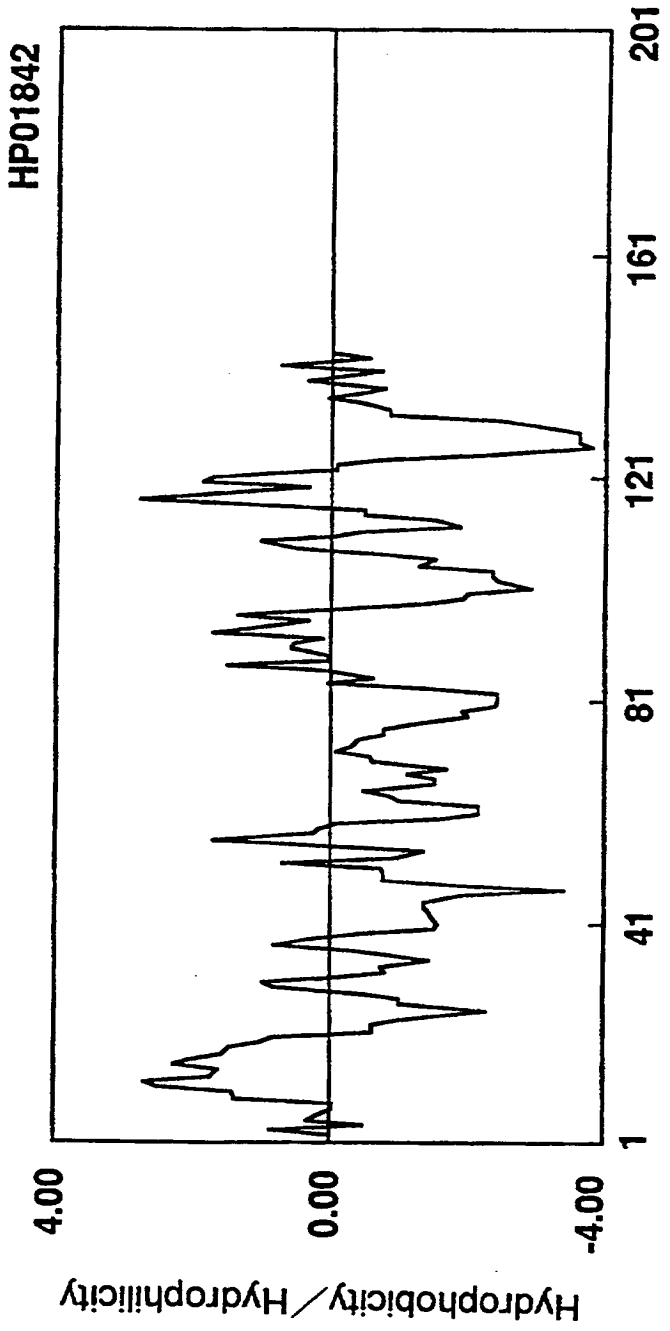

Fig. 3

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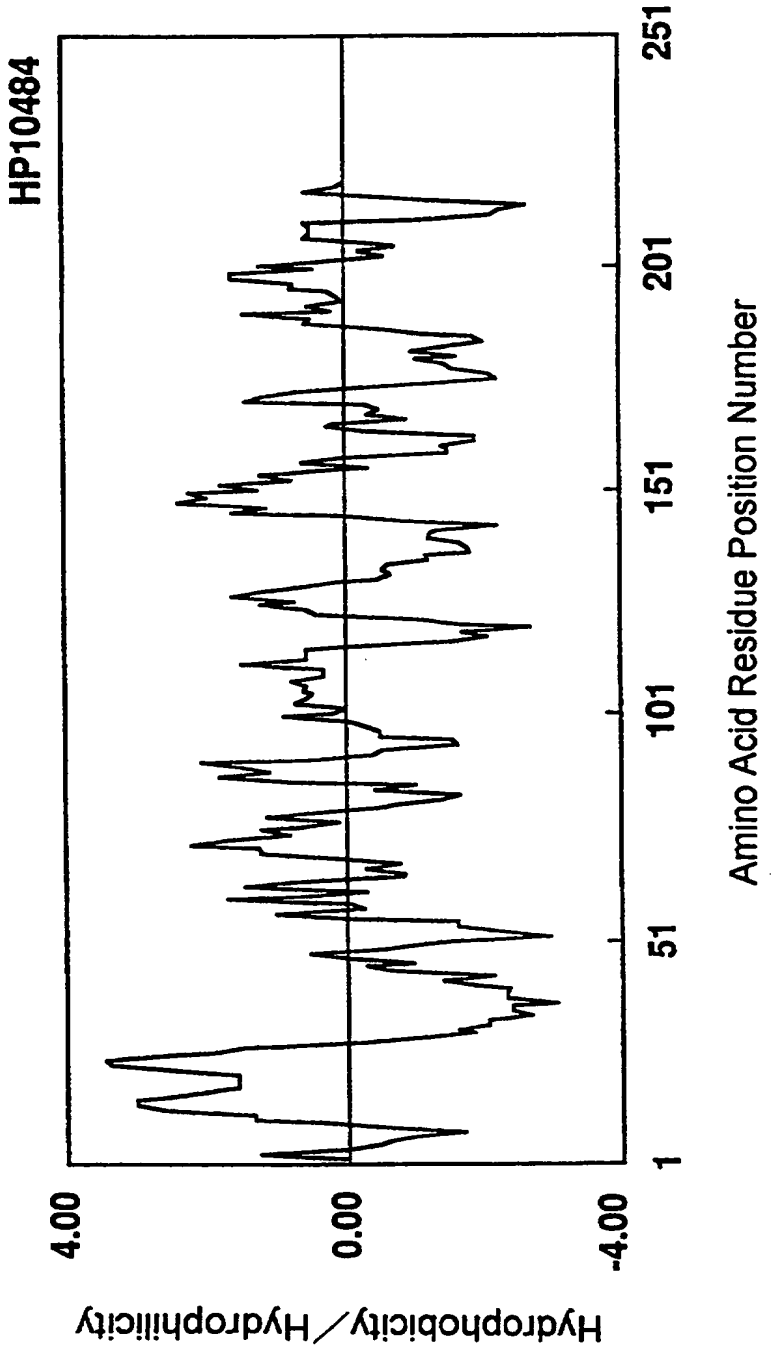

Fig. 4

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Sequence Listing

<110> Sagami Chemical Research Center

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<140>

<141>

<150> Japan 9-276268

<151> 1997-10-08

<160> 16

<170> Windows 95 (Word 98)

<210> 1

<211> 167

<212> PRT

<213> Homo sapiens

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35 40 45

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50 55 60

Gln Val Arg Tyr Gly Lys Val Trp Ser Asp Tyr Val Gly Gly Arg Asn

65 70 75 80

Gly Asp Leu Glu Glu Ile Phe Leu His Pro Gly Glu Ser Val Ile Gln

85 90 95

Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr

100 105 110

Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe

115 120 125

Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly

130 135 140

Arg Ser Gly Ser Leu Ile Asp Ala Ile Gly Leu His Trp Asp Val Tyr

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Pro Thr Ser Cys Ser Arg Cys

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<211> 172

<212> PRT

<213> Homo sapiens

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35 40 45
Glu Ala Ala Ala Ser Gly Leu Pro Leu Met Val Ile Ile His Lys Ser
50 55 60
Trp Cys Gly Ala Cys Lys Ala Leu Lys Pro Lys Phe Ala Glu Ser Thr
65 70 75 80
Glu Ile Ser Glu Leu Ser His Asn Phe Val Met Val Asn Leu Glu Asp
85 90 95
Glu Glu Glu Pro Lys Asp Glu Asp Phe Ser Pro Asp Gly Gly Tyr Ile
100 105 110
Pro Arg Ile Leu Phe Leu Asp Pro Ser Gly Lys Val His Pro Glu Ile
115 120 125
Ile Asn Glu Asn Gly Asn Pro Ser Tyr Lys Tyr Phe Tyr Val Ser Ala
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Glu Gln Val Val Gln Gly Met Lys Glu Ala Gln Glu Arg Leu Thr Gly
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165 170

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Gln Pro Gly Ser Met Gly Leu Asp Lys Asn Thr Val His Asp Gln Glu

35 40 45

His Ile Met Glu His Leu Glu Gly Val Ile Asn Lys Pro Glu Ala Glu

50 55 60

Met Ser Pro Gln Glu Leu Gln Leu His Tyr Phe Lys Met His Asp Tyr

65 70 75 80

Asp Gly Asn Asn Leu Leu Asp Gly Leu Glu Leu Ser Thr Ala Ile Thr

85 90 95

His Val His Lys Glu Glu Gly Ser Glu Gln Ala Pro Leu Met Ser Glu

100 105 110

Asp Glu Leu Ile Asn Ile Ile Asp Gly Val Leu Arg Asp Asp Asp Lys

115 120 125

Asn Asn Asp Gly Tyr Ile Asp Tyr Ala Glu Phe Ala Lys Ser Leu Gln

130 135 140

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<211> 220

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50 55 60
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65 70 75 80
Arg Gly Arg Pro Phe Ala Asp Val Leu Ser Leu Ser Asp Gly Pro Pro
85 90 95
Gly Ala Gly Ser Gly Val Pro Tyr Phe Tyr Leu Ser Pro Leu Gln Leu
100 105 110
Ser Val Ser Asn Leu Gln Glu Asn Pro Tyr Ala Thr Leu Thr Met Thr
115 120 125
Leu Ala Gln Thr Asn Phe Cys Lys Lys His Gly Phe Asp Pro Gln Ser
130 135 140
Pro Leu Cys Val His Ile Met Leu Ser Gly Thr Val Thr Lys Val Asn
145 150 155 160
Glu Thr Glu Met Asp Ile Ala Lys His Ser Leu Phe Ile Arg His Pro
165 170 175
Glu Met Lys Thr Trp Pro Ser Ser His Asn Trp Phe Phe Ala Lys Leu
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tctggcaacc agttggacgg ccccatcacc gccctccggg tccgagtcaa cacatactac 180

atcgtaggtc ttcaggtgcg ctatggcaag gtgtggagcg actatgtggg tggtcgcaac 240

ggagacctgg aggagatctt tctgcacccc ggggaatcag tgatccaggt ttctgggaag 300

tacaagtggt acctgaagaa gctggtattt gtgacagaca agggccgcta tctgtctttt 360

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ttcatcagtg gccggtctgg tttctctcatc gatgccattg gcctgcactg ggatgtttac 480

cccactagct gcagcagatg c 501

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aggacactgg aagatgggaa gaaagaagca gctgccagtg gactgcccct gatggtgatt 180

attcataaat cctggtgtgg agcttgcaaa gctctaaagc ccaaatttgc agaatctacg 240

gaaatttcag aactctccca taattttgtt atggtaaatc ttgaggatga agaggaaccc 300

aaagatgaag atttcagccc tgacgggggt tatattccac gaatcctttt tctggatccc 360

agtggcaagg tgcatcctga aatcatcaat gagaatggaa accccagcta caagtatttt 420

tatgtcagtg ccgagcaagt tgttcagggg atgaaggaag ctcaggaaag gctgacgggt 480

gatgccttca gaaagaaaca tcttgaagat gaattg 516

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aagaacacag tgcacgacca agagcatatc atggagcatc tagaaggtgt catcaacaaa 180

ccagaggcgg agatgtcgcc acaagaattg cagctccatt acttcaaaat gcatgattat 240

gatggcaata atttgcttga tggcttagaa crctccacag ccatcactca tgtccataag 300

gaggaaggga gtgaacaggc accactaatg agtgaagatg aactgattaa cataatagat 360

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ctgttggcgc tgctcgtgtc gcccgcgcgg ggtcgcggcg gccgggacca cggggactgg 120

gacgaggcct cccggctgcc gccgctacca ccccgcgagg acgcggcgcg cgtggcccgc 180

ttcgtgacgc acgtctccga ctggggcgct ctggccacca tctccacgct ggaggcggtg 240

cgcggccggc ccttcgccga cgtcctctcg ctcagcgacg ggcccccggg cgcgggcagc 300

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gaaacagaaa tggatattgc aaagcattcg ttattcattc gacaccctga gatgaaaacc 540

tggccttcca gccataattg gttctttgct aagttgaata taaccaatat ctgggtcctg 600

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<213> Homo sapiens

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Met Leu Thr Val Ala Leu Leu Ala Leu Leu Cys Ala Ser Ala Ser Gly

1 5 10 15

aat gcc att cag gcc agg tct tcc tcc tat agt gga gag tat gga agt 154

Asn Ala Ile Gln Ala Arg Ser Ser Ser Tyr Ser Gly Glu Tyr Gly Ser

20 25 30

ggt ggt gga aag cga ttc tct cat tct ggc aac cag ttg gac ggc ccc 202

Gly Gly Gly Lys Arg Phe Ser His Ser Gly Asn Gln Leu Asp Gly Pro

35 40 45

atc acc gcc ctc cgg gtc cga gtc aac aca tac tac atc gta ggt ctt 250

Ile Thr Ala Leu Arg Val Arg Val Asn Thr Tyr Tyr Ile Val Gly Leu

50 55 60

cag gtg cgc tat ggc aag gtg tgg agc gac tat gtg ggt ggt cgc aac 298

Gln Val Arg Tyr Gly Lys Val Trp Ser Asp Tyr Val Gly Gly Arg Asn

65 70 75 80

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85 90 95

gtt tct ggg aag tac aag tgg tac ctg aag aag ctg gta ttt gtg aca 394
Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr
100 105 110

gac aag ggc cgc tat ctg tct ttt ggg aaa gac agt ggc aca agt ttc 442
Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe
115 120 125

aat gcc gtc ccc ttg cac ccc aac acc gtg ctc cgc ttc atc agt ggc 490
Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly
130 135 140

cgg tct ggt tct ctc atc gat gcc att ggc ctg cac tgg gat gtt tac 538
Arg Ser Gly Ser Leu Ile Asp Ala Ile Gly Leu His Trp Asp Val Tyr
145 150 155 160

ccc act agc tgc agc aga tgc tgagcctcct ctccttggca ggggcactgt g 590
Pro Thr Ser Cys Ser Arg Cys
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Gly Gly Gly Lys Arg Phe Ser His Ser Gly Asn Gln Leu Asp Gly Pro
35 40 45
Ile Thr Ala Leu Arg Val Arg Val Asn Thr Tyr Tyr Ile Val Gly Leu
50 55 60
Gln Val Arg Tyr Gly Lys Val Trp Ser Asp Tyr Val Gly Gly Arg Asn
65 70 75 80
Gly Asp Leu Glu Glu Ile Phe Leu His Pro Gly Glu Ser Val Ile Gln
85 90 95
Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr
100 105 110
Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe
115 120 125
Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly
130 135 140
Arg Ser Gly Ser Leu Ile Asp Ala Ile Gly Leu His Trp Asp Val Tyr
145 150 155 160
Pro Thr Ser Cys Ser Arg Cys
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<213> Homo sapiens

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gtccgctcag ccccgtggcc ccgcgcacct actgcc atg gag acg cgg cct cgt 114
Met Glu Thr Arg Pro Arg
1 5

ctc ggg gcc acc tgt ttg ctg ggc ttc agt ttc ctg ctc ctc gtc atc 162
Leu Gly Ala Thr Cys Leu Leu Gly Phe Ser Phe Leu Leu Leu Val Ile
10 15 20

tct tct gat gga cat aat ggg ctt gga aag ggt ttt gga gat cat att 210
Ser Ser Asp Gly His Asn Gly Leu Gly Lys Gly Phe Gly Asp His Ile
25 30 35

cat tgg agg aca ctg gaa gat ggg aag aaa gaa gca gct gcc agt gga 258
His Trp Arg Thr Leu Glu Asp Gly Lys Lys Glu Ala Ala Ala Ser Gly
40 45 50

ctg ccc ctg atg gtg att att cat aaa tcc tgg tgt gga gct tgc aaa 306
Leu Pro Leu Met Val Ile Ile His Lys Ser Trp Cys Gly Ala Cys Lys
55 60 65 70

gct cta aag ccc aaa ttt gca gaa tct acg gaa att tca gaa ctc tcc 354
Ala Leu Lys Pro Lys Phe Ala Glu Ser Thr Glu Ile Ser Glu Leu Ser
75 80 85

cat aat ttt gtt atg gta aat ctt gag gat gaa gag gaa ccc aaa gat 402
His Asn Phe Val Met Val Asn Leu Glu Asp Glu Glu Glu Pro Lys Asp
90 95 100

gaa gat ttc agc cct gac ggg ggt tat att cca cga atc ctt ttt ctg 450
Glu Asp Phe Ser Pro Asp Gly Gly Tyr Ile Pro Arg Ile Leu Phe Leu
105 110 115

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Pro Ser Tyr Lys Tyr Phe Tyr Val Ser Ala Glu Gln Val Val Gln Gly

135 140 145 150

atg aag gaa gct cag gaa agg ctg acg ggt gat gcc ttc aga aag aaa 594

Met Lys Glu Ala Gln Glu Arg Leu Thr Gly Asp Ala Phe Arg Lys Lys

155 160 165

cat ctt gaa gat gaa ttg taacatgaat gtgccccttc tttcatcaga gttagtgt 650

His Leu Glu Asp Glu Leu

170

tctggaagga aagcagcagg gaagggaata ttgaggaatc atctagaaca attaagccga 710

ccaggaaacc tcattcctac ctacactgga aggagcgctc tcactgtgga agagttctgc 770

taacagaagc tggtctgcat gtttgtggat ccagcggaga gtggcagact ttcttctcct 830

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aaaacttgag ccacttggat gtttactcct cgcacttaag tatttgagtc ttttcccatt 950

tcctcccact ttactcacct tagtggtgaa aggagactag tagcatcttt tctacaacgt 1010

taaaattgca gaagtagctt atcattaaaa aacaacaaca acaacaataa caataaatcc 1070

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tctcctggga ataattgtgg gcttcttccc aaatttctac agcctctttc ctcttctcat 1190

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25 30 35

His Trp Arg Thr Leu Glu Asp Gly Lys Lys Glu Ala Ala Ala Ser Gly
40 45 50

Leu Pro Leu Met Val Ile Ile His Lys Ser Trp Cys Gly Ala Cys Lys
55 60 65 70

Ala Leu Lys Pro Lys Phe Ala Glu Ser Thr Glu Ile Ser Glu Leu Ser
75 80 85

His Asn Phe Val Met Val Asn Leu Glu Asp Glu Glu Glu Pro Lys Asp
90 95 100

Glu Asp Phe Ser Pro Asp Gly Gly Tyr Ile Pro Arg Ile Leu Phe Leu
105 110 115

Asp Pro Ser Gly Lys Val His Pro Glu Ile Ile Asn Glu Asn Gly Asn
120 125 130

Pro Ser Tyr Lys Tyr Phe Tyr Val Ser Ala Glu Gln Val Val Gln Gly
135 140 145 150

Met Lys Glu Ala Gln Glu Arg Leu Thr Gly Asp Ala Phe Arg Lys Lys
155 160 165

His Leu Glu Asp Glu Leu
170

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<213> Homo sapiens

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Met Arg Ser Leu Leu Arg Thr Pro Phe
1 5

ctg tgt ggc ctg ctc tgg gcc ttt tgt gcc cca ggc gcc agg gct gag 159
Leu Cys Gly Leu Leu Trp Ala Phe Cys Ala Pro Gly Ala Arg Ala Glu
10 15 20 25

gag cct gca gcc agc ttc tcc caa ccc ggc agc atg ggc ctg gat aag 207
Glu Pro Ala Ala Ser Phe Ser Gln Pro Gly Ser Met Gly Leu Asp Lys
30 35 40

aac aca gtg cac gac caa gag cat atc atg gag cat cta gaa ggt gtc 255
Asn Thr Val His Asp Gln Glu His Ile Met Glu His Leu Glu Gly Val
45 50 55

atc aac aaa cca gag gcg gag atg tcg cca caa gaa ttg cag ctc cat 303
Ile Asn Lys Pro Glu Ala Glu Met Ser Pro Gln Glu Leu Gln Leu His
60 65 70

tac ttc aaa atg cat gat tat gat ggc aat aat ttg ctt gat ggc tta 351
Tyr Phe Lys Met His Asp Tyr Asp Gly Asn Asn Leu Leu Asp Gly Leu
75 80 85

gaa ctc tcc aca gcc atc act cat gtc cat aag gag gaa ggg agt gaa 399

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Gln Ala Pro Leu Met Ser Glu Asp Glu Leu Ile Asn Ile Ile Asp Gly

110 115 120

gtt ttg aga gat gat gac aag aac aat gat gga tac att gac tat gct 495

Val Leu Arg Asp Asp Asp Lys Asn Asn Asp Gly Tyr Ile Asp Tyr Ala

125 130 135

gaa ttt gca aaa tca ctg cag tagatgttat ttggccatct cctggttata taca 550

Glu Phe Ala Lys Ser Leu Gln

140

aatgtgaccc gtgataatgt gattgaacac tttagtaatg caaaat 596

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<211> 144

<212> PRT

<213> Homo sapiens

<400> 14

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1 5

Leu Cys Gly Leu Leu Trp Ala Phe Cys Ala Pro Gly Ala Arg Ala Glu

10 15 20 25

Glu Pro Ala Ala Ser Phe Ser Gln Pro Gly Ser Met Gly Leu Asp Lys

30 35 40

Asn Thr Val His Asp Gln Glu His Ile Met Glu His Leu Glu Gly Val

45 50 55

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60 65 70
Tyr Phe Lys Met His Asp Tyr Asp Gly Asn Asn Leu Leu Asp Gly Leu
75 80 85
Glu Leu Ser Thr Ala Ile Thr His Val His Lys Glu Glu Gly Ser Glu
90 95 100 105
Gln Ala Pro Leu Met Ser Glu Asp Glu Leu Ile Asn Ile Ile Asp Gly
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Glu Phe Ala Lys Ser Leu Gln
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Gly Leu Ser Arg Gly Ser Ala Arg Ala Leu Leu Ala Ala Leu Leu Ala
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agc aac ctg cag gag aat cca tat gct aca ctg acc atg act ttg gca 442
Ser Asn Leu Gln Glu Asn Pro Tyr Ala Thr Leu Thr Met Thr Leu Ala
115 120 125 130

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Gln Thr Asn Phe Cys Lys Lys His Gly Phe Asp Pro Gln Ser Pro Leu
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Cys Val His Ile Met Leu Ser Gly Thr Val Thr Lys Val Asn Glu Thr
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acc aat atc tgg gtc ctg gac tac tt tggtggacca aaaatcgtg 679
Thr Asn Ile Trp Val Leu Asp Tyr Phe GlyGlyPro LysIleVal
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Thr Pro Glu Glu Tyr Tyr Asn Val Thr Val Gln
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Pro Arg Glu Asp Ala Ala Arg Val Ala Arg Phe Val Thr His Val Ser
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Asp Trp Gly Ala Leu Ala Thr Ile Ser Thr Leu Glu Ala Val Arg Gly
70 75 80
Arg Pro Phe Ala Asp Val Leu Ser Leu Ser Asp Gly Pro Pro Gly Ala
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Gly Ser Gly Val Pro Tyr Phe Tyr Leu Ser Pro Leu Gln Leu Ser Val
100 105 110
Ser Asn Leu Gln Glu Asn Pro Tyr Ala Thr Leu Thr Met Thr Leu Ala
115 120 125 130
Gln Thr Asn Phe Cys Lys Lys His Gly Phe Asp Pro Gln Ser Pro Leu
135 140 145
Cys Val His Ile Met Leu Ser Gly Thr Val Thr Lys Val Asn Glu Thr
150 155 160
Glu Met Asp Ile Ala Lys His Ser Leu Phe Ile Arg His Pro Glu Met
165 170 175
Lys Thr Trp Pro Ser Ser His Asn Trp Phe Phe Ala Lys Leu Asn Ile
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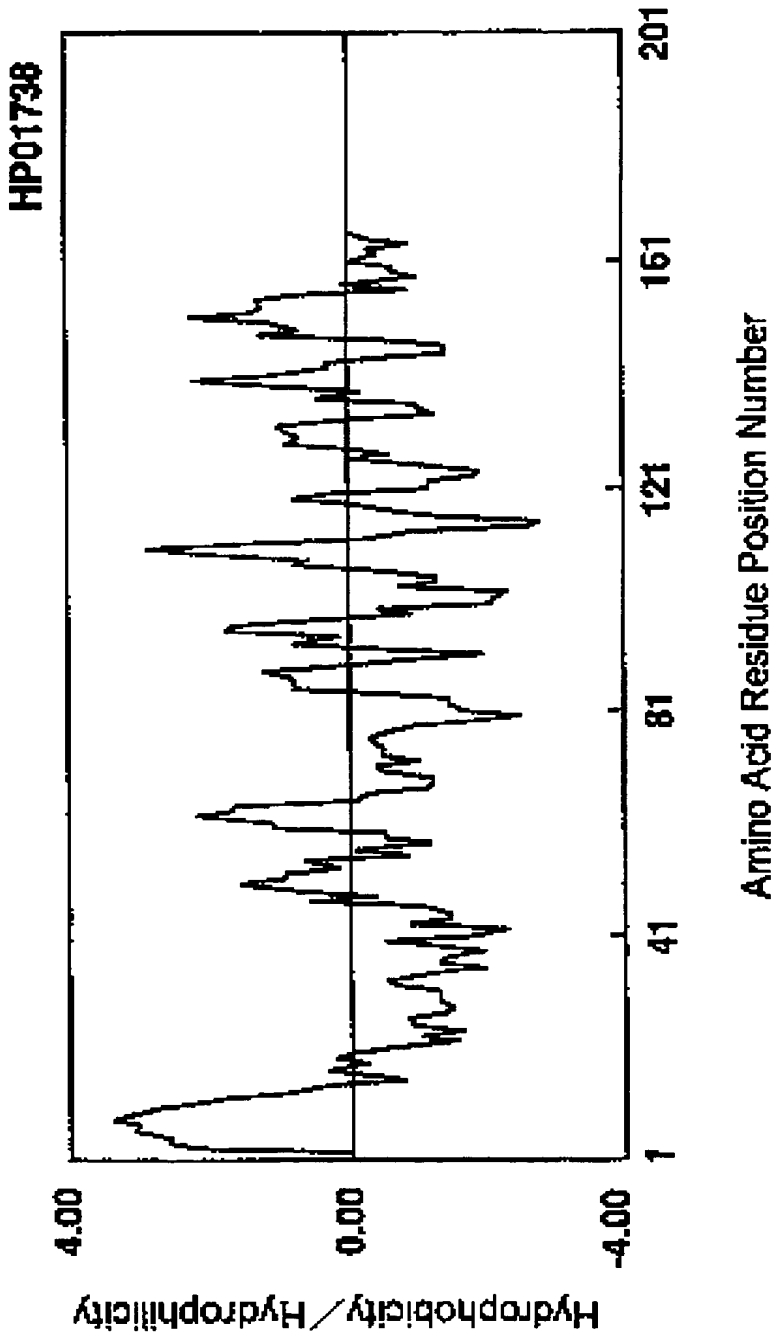

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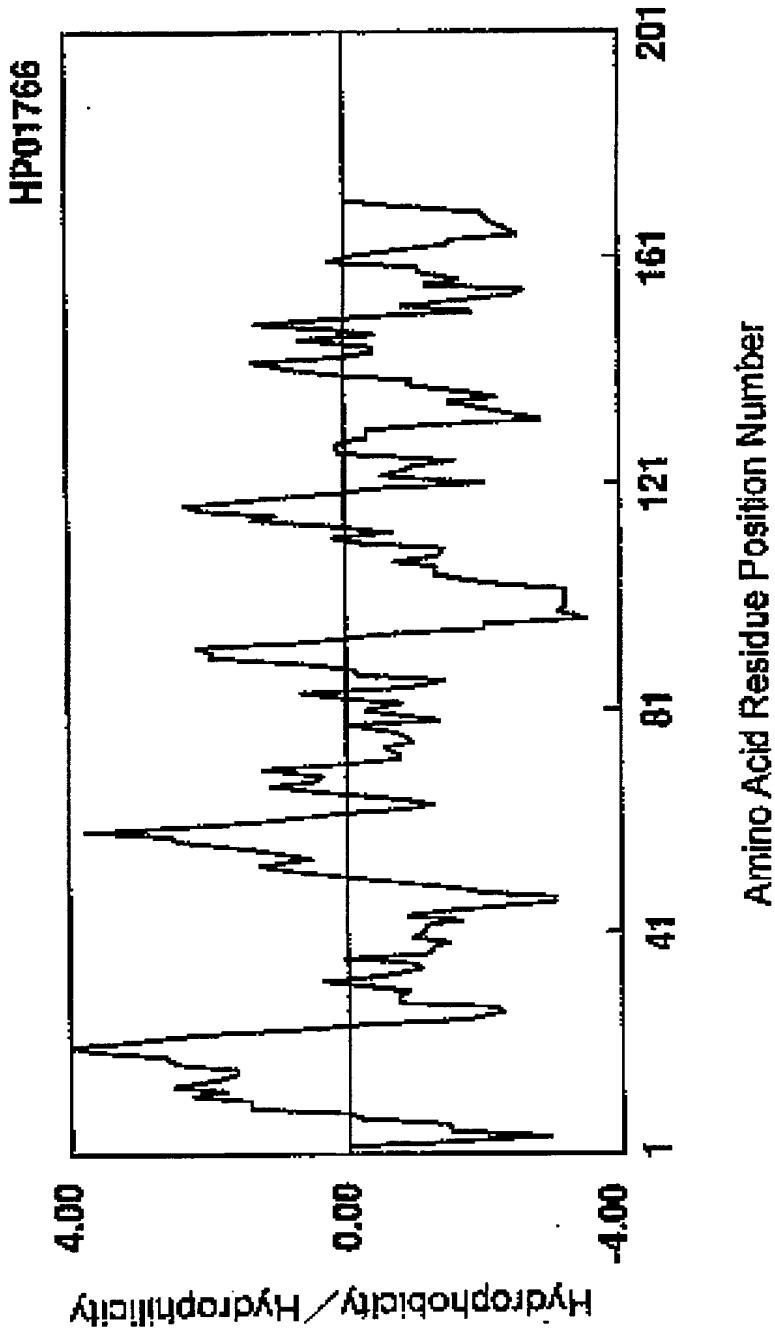

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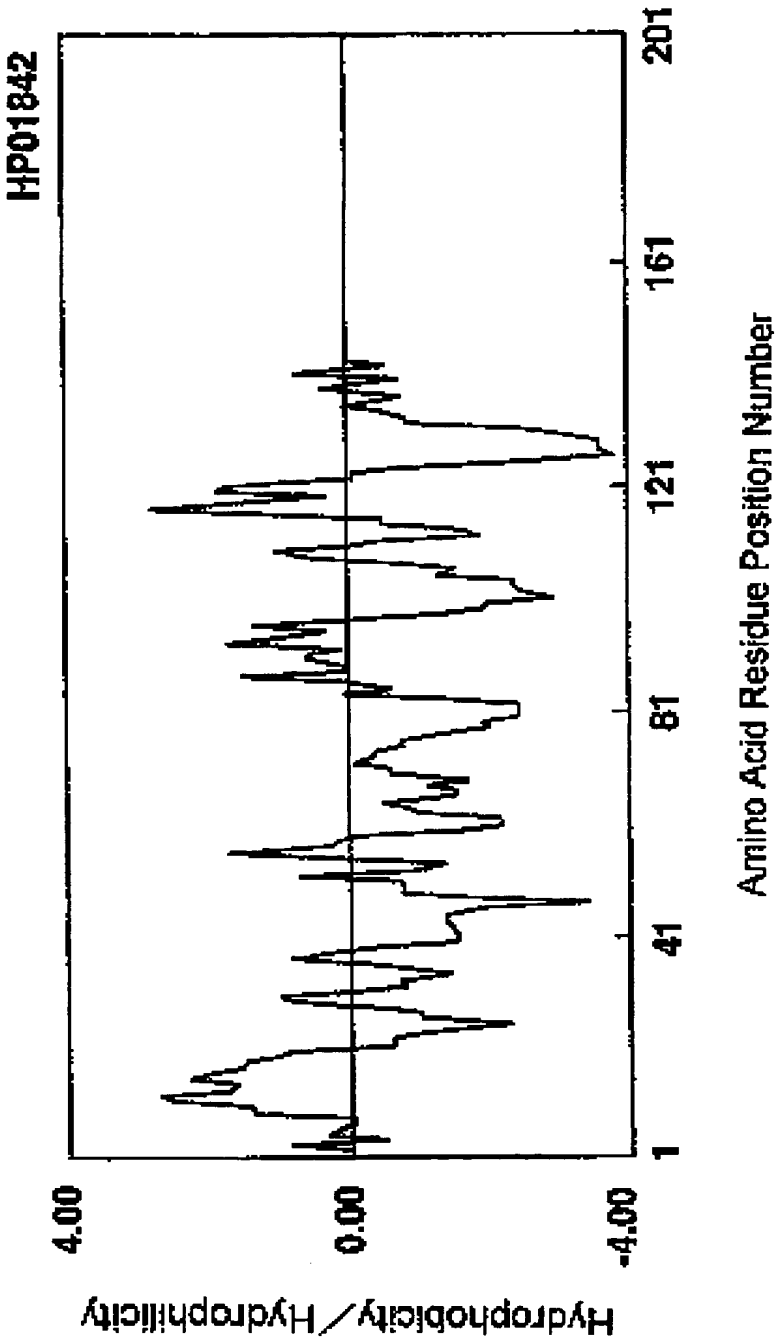

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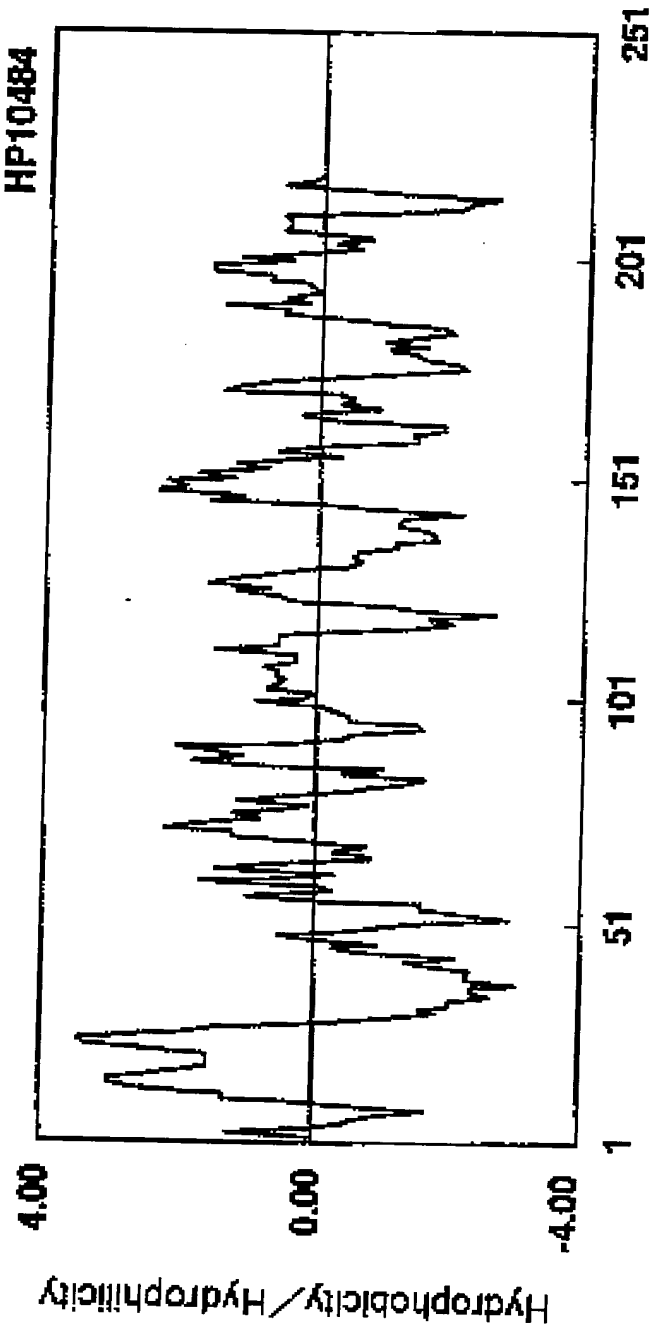

Fig. 4

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50 55 60

Gln Val Arg Tyr Gly Lys Val Trp Ser Asp Tyr Val Gly Gly Arg Asn

65 70 75 80

Gly Asp Leu Glu Glu Ile Phe Leu His Pro Gly Glu Ser Val Ile Gln

85 90 95

Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr

100 105 110

Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe

115 120 125

Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly

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Trp Cys Gly Ala Cys Lys Ala Leu Lys Pro Lys Phe Ala Glu Ser Thr
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Glu Ile Ser Glu Leu Ser His Asn Phe Val Met Val Asn Leu Glu Asp
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Glu Glu Glu Pro Lys Asp Glu Asp Phe Ser Pro Asp Gly Gly Tyr Ile
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Pro Arg Ile Leu Phe Leu Asp Pro Ser Gly Lys Val His Pro Glu Ile
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50 55 60

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65 70 75 80

Asp Gly Asn Asn Leu Leu Asp Gly Leu Glu Leu Ser Thr Ala Ile Thr

85 90 95

His Val His Lys Glu Glu Gly Ser Glu Gln Ala Pro Leu Met Ser Glu

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Arg Gly Arg Pro Phe Ala Asp Val Leu Ser Leu Ser Asp Gly Pro Pro
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Glu Thr Glu Met Asp Ile Ala Lys His Ser Leu Phe Ile Arg His Pro
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Glu Met Lys Thr Trp Pro Ser Ser His Asn Trp Phe Phe Ala Lys Leu
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attcataaat cctggtgtgg agcttgcaaa gctctaaagc ccaaatttgc agaatctacg 240

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35 40 45

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Ile Thr Ala Leu Arg Val Arg Val Asn Thr Tyr Tyr Ile Val Gly Leu

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cag gtg cgc tat ggc aag gtg tgg agc gac tat gtg ggt ggt cgc aac 298

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Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr
            100                 105                 110

gac aag ggc cgc tat ctg tct ttt ggg aaa gac agt ggc aca agt ttc      442
Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe
        115                 120                 125

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Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly
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cgg tct ggt tct ctc atc gat gcc att ggc ctg cac tgg gat gtt tac      538
Arg Ser Gly Ser Leu Ile Asp Ala Ile Gly Leu His Trp Asp Val Tyr
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Glu Val Arg Tyr Gly Lys Val Trp Ser Asp Tyr Val Gly Gly Arg Asn
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Gly Asp Leu Glu Glu Ile Phe Leu His Pro Gly Glu Ser Val Ile Gln
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Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr
100 105 110

Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe
115 120 125

Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly
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Met Glu Thr Arg Pro Arg

1 5

ctc ggg gcc acc tgt ttg ctg ggc ttc agt ttc ctg ctc ctc gtc atc 162

Leu Gly Ala Thr Cys Leu Leu Gly Phe Ser Phe Leu Leu Leu Val Ile

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Ser Ser Asp Gly His Asn Gly Leu Gly Lys Gly Phe Gly Asp His Ile

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cat tgg agg aca ctg gaa gat ggg aag aaa gaa gcu gct gcc agt gga 258

His Trp Arg Thr Leu Glu Asp Gly Lys Lys Glu Ala Ala Ala Ser Gly

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Leu Pro Leu Met Val Ile Ile His Lys Ser Trp Cys Gly Ala Cys Lys

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gct ctc aag ccc aaa ttt gca gaa tct acg gaa att tca gaa ctc tcc 354

Ala Leu Lys Pro Lys Phe Ala Glu Ser Thr Glu Ile Ser Glu Leu Ser

75 80 85

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90 95 100

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Glu Asp Phe Ser Pro Asp Gly Gly Tyr Ile Pro Arg Ile Leu Phe Leu

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135 140 145 150

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Met Lys Glu Ala Gln Glu Arg Leu Thr Gly Asp Ala Phe Arg Lys Lys
155 160 165

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His Leu Glu Asp Glu Leu
170

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25 30 35

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40 45 50

Leu Pro Leu Met Val Ile Ile His Lys Ser Trp Cys Gly Ala Cys Lys
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Ala Leu Lys Pro Lys Phe Ala Glu Ser Thr Glu Ile Ser Glu Leu Ser
75 80 85

His Asn Phe Val Met Val Asn Leu Glu Asp Glu Glu Glu Pro Lys Asp
90 95 100

Glu Asp Phe Ser Pro Asp Gly Gly Tyr Ile Pro Arg Ile Leu Phe Leu
105 110 115

Asp Pro Ser Gly Lys Val His Pro Glu Ile Ile Asn Glu Asn Gly Asn
120 125 130

Pro Ser Tyr Lys Tyr Phe Tyr Val Ser Ala Glu Gln Val Val Gln Gly
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Met Arg Ser Leu Leu Arg Thr Pro Phe
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<400> 16

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70 75 80
Arg Pro Phe Ala Asp Val Leu Ser Leu Ser Asp Gly Pro Pro Gly Ala
85 90 95
Gly Ser Gly Val Pro Tyr Phe Tyr Leu Ser Pro Leu Gln Leu Ser Val
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A3

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(81) Designated States: AU, CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: HUMAN PROTEINS HAVING SECRETORY SIGNAL SEQUENCES AND cDNAS ENCODING THESE PROTEINS

(57) Abstract

The invention provides human proteins having secretory signal sequences and DNAs coding for these proteins as well as eucaryotic cells forming said proteins by secretory expression. All of the proteins are secreted outside the cells and exist in the extracellular liquid or on the cell membrane, so that they are considered to be proteins controlling the proliferation and the differentiation of the cells. Accordingly, the proteins can be employed as pharmaceuticals or as antigens for preparing antibodies against said proteins. Furthermore, said DNAs can be utilized for expression of said proteins in large amounts. Eucaryotic cells wherein expression vectors of said cDNAs are introduced can be utilized for secretory production of the proteins encoded by said cDNAs.

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BNSDOCID: <WO 9918204A3 >

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/JP 98/04476

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YOKOYAMA-KOBAYASHI M ET AL: "A SIGNAL SEQUENCE DETECTION SYSTEM USING SECRETED PROTEASE ACTIVITY AS AN INDICATOR" GENE, vol. 163, 1995, pages 193-196, XP002046435 see the whole document ---	
A	TASHIRO K ET AL: "SIGNAL SEQUENCE TRAP: A CLONING STRATEGY FOR SECRETED PROTEINS AND TYPE I MEMBRANE PROTEINS" SCIENCE, vol. 261, 30 July 1993, pages 600-603, XP000673204 cited in the application see the whole document ---	
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 1999	03. 06. 99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Odèrwald, H

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INTERNATIONAL SEARCH REPORT

International Application No
CT/JP 98/04476

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39419 A (HUMAN GENOME SCIENCES INC ;YU GUO LIANG (US); ROSEN CRAIG A (US)) 12 December 1996 see abstract; claims 1,4,5,8; figure 6; example 4 ---	1-6
E	WO 98 44160 A (ABBOTT LAB) 8 October 1998 see SEQ ID NO. 8 and 20. see abstract; claims 11,12,15-18,30,38,39 see SEQ ID NO.8 (page 91) and SEQ ID NO. 20 (page 94) -----	1-6

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

BNSDOCID: <WO... 99182043A1>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 98/04476

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

See Subject 1, extra sheet.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BNSDOCID: <WO 9918204A3_I_>
BNSDOCID: <WO 9918204A3 I >

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-6 all partially

Protein comprising SEQ ID NO. 1, DNAs related to said protein represented by SEQ ID NO. 5 and 9, vector expressing said DNA, cell expressing said DNA.

2. Claims: 1-6 all partially

Protein comprising SEQ ID NO. 2, DNAs related to said protein represented by SEQ ID NO. 6 and 11, vector expressing said DNA, cell expressing said DNA.

3. Claims: 1-6 all partially

Protein comprising SEQ ID NO. 3, DNAs related to said protein represented by SEQ ID NO. 7 and 13, vector expressing said DNA, cell expressing said DNA.

4. Claims: 1-6 all partially

Protein comprising SEQ ID NO. 4, DNAs related to said protein represented by SEQ ID NO. 8 and 15, vector expressing said DNA, cell expressing said DNA.

BNSDOCID: WO 9948894A2A1 >

INTERNATIONAL SEARCH REPORT

[redacted]on on patent family members

International Application No
[redacted]/JP 98/04476

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639419 A	12-12-1996	CA 2221798 A	12-12-1996
		US 5733748 A	31-03-1998
		AU 2820595 A	24-12-1996
		EP 0847398 A	17-06-1998
WO 9844160 A	08-10-1998	NONE	

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-PNSDOCID <WO 9918294A3 | >

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